EDGEWOOD

RESEARCH, DEVELOPMENT & ENGINEERING CENTER

U.S. ARMY SOLDIER AND BIOLOGICAL CHEMICAL COMMAND

ECBC-TN-001

TEST PROCEDURE FOR REMOVING POLYSTYRENE LATEX MICROSPHERES FROM MEMBRANE FILTERS

Jana Kesavanathan
Robert W. Doherty

RESEARCH AND TECHNOLOGY DIRECTORATE

July 1999

Approved for public release; distribuiton is unlimited.

CUM SCIENTIA DEFENDIMUS

Aberdeen Proving Ground, MD 21010-5424

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1999 July	Final, 98 Sep – Oct 98

4. TITLE AND SUBTITLE
Test Procedure for Removing Polystyrene Latex Microspheres from Membrane Filters

5. FUNDING NUMBERS
PR-622384/ACB2

6. AUTHOR(S)
Kesavanathan,* Jana; and Doherty, Robert W.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
DIR, ECBC,** ATTN: AMSSB-RRT-TA, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TN-001

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
*Independent contractor working for Aerosol Sciences Team, Research and Technology Directorate, funded under Contract No. DAAD13-98-M-0083.
**When this work was conducted, the U.S. Army Edgewood Chemical Biological Center (ECBC) was known as the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC).

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited .

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 words)***

A test procedure was developed for removing 1μm polystyrene latex (PSL) microspheres from membrane filters. The procedure consisted of placing the filter, with the collected PSL microspheres, in a test tube with water and glass beads. The text tube was held on a padded shaker table to induce vortexing for 50 sec and then was shaken by hand for 10 sec. Vortexing and hand shaking were repeated many times, and samples were taken after predetermined times of shaking. The samples were analyzed using a fluorometer. From the results, a total shake time of 5 min (50 sec vortexing and 10 sec hand shaking repeated five times) was adapted as our shaking procedure for removing the PSL microspheres from the membrane filters. No significant differences in the recovery were found when the procedure was followed by two test operators.

14. SUBJECT TERMS
Polystyrene latex microspheres
Sample recovery
Membrane filter

15. NUMBER OF PAGES
13

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z3918

Blank

PREFACE

The work described in this report was authorized under Project No. 622384/ACB2, Non-Medical CB Defense. The work was started in September 1998 and completed in October 1998.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for release to the public. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Blank

CONTENTS

Blank

TEST PROCEDURE FOR REMOVING POLYSTYRENE LATEX MICROSPHERES FROM MEMBRANE FILTERS

1. INTRODUCTION

Filters are often used to collect airborne particles, such as atmospheric aerosols or test particles used in chambers or controlled wind tunnel experiments. Procedures for removing the particles from the filters, however, are often not described or are generated separately for each test. Because of the lack of controlled removal procedures, a series of tests were performed with the objective of developing a more formal particle removal procedure.

Objectives of the study, therefore, are (1) to develop a standard procedure for the removal of particles from membrane filters and (2) to test the variability in the results between two test operators who followed the same procedure. The type of particles selected for the study were polystyrene latex (PSL) microspheres that were collected on membrane filters and analyzed using a fluorometer.

2. APPROACH

2.1. Sample Preparation.

The microspheres selected for the study were blue fluorescing, mono-disperse, polymer microspheres with a diameter of 0.988 μm and were obtained from Duke Scientific Corp. (catalog no. B0100B). The microspheres, as supplied from the vendor, were contained in an aqueous solution with a proprietary surfactant. The filters selected for the study were 47-mm diameter, polycarbonate membrane filters manufactured by Poretics Corp. (catalog no. 13043). The small pore size (0.6 μm) of the filter ensured that the microspheres were captured on the filter surface.

A solution of microspheres was prepared by adding 6 drops from the vendor's solution containing the microspheres to 20 m1 of deionized distilled water. A known mass of liquid containing the microspheres was removed from the 20 ml supply using a disposable pipette and placed on the test filter. The mass of liquid containing the microspheres, which was deposited on the filter, was determined by differential weighing pipette before and after dispersing the solution. Mass measurements were made using a balance with an accuracy of ±20 μg.

The filter was next dried by passing air through the filter. The dry filter was then placed into a plastic, disposable 50 ml centrifuge tube with screw cap. The tube contained 15 m1 of deionized distilled water and 6 – 10 glass beads (approximately 3.0 mm diameter). The glass beads filled the lower conical section of the test tube. The test tube was then shaken. The shaking procedure consisted of holding the test tube on a padded shaker platform (Maxi-Mix shaker, Type 16700), which induced a vortex motion. The procedure called for 50 sec of mechanical shaking followed by 10 sec of manual shaking. The reference samples were prepared by transferring a known mass of liquid from the previously prepared 20 ml supply directly to the plastic disposable 50 ml centrifuge tubes. After the shaking procedure was completed, test samples were prepared by transferring the liquid in the test tube to small disposable glass culture tubes (10 mm x 75 mm). Twelve test samples were prepared plus six reference samples.

A relative measure of the quantity of microspheres in the small culture tube was determined by using a fluorometer (Sequoia Turner Model 45) with a narrow band excitation filter NB 440[1] and an emission filter SC 515[2]. The fluorescent measurements were made after zeroing the instrument at the selected gain setting. Measurements were taken with gain settings of 10 and 50. No attempt was made to convert the fluorometer readings to an absolute number count.

2.2. Experiments.

Experiment 1. The shaking sequence described above was repeated for total shake times of 1 to 6 min to determine a reasonable shaking time for removing most of the microspheres from filters.

Experiment 2. A second experiment was conducted to determine the effect that two different experimentalists might have on the results and to measure instrument variability. Two operators performed the shaking sequence for 5 min. Each operator examined 4 filters. The fluorometer measurements were made with gains set at 10 and 50. The data obtained from Experiment 2, therefore, provided a means for evaluating both operator effects and gain settings for the fluorometer.

3. RESULTS

3.1. Experiment 1, Determination of Optimum Shaking Time.

The fluorometer readings taken for different shake time are summarized in Table 1 and plotted in Figure 1. The data were normalized by dividing the fluorometer measurements by the gain setting and the mass of liquid (containing the microspheres) on the filter. The normalized data for the shake times for two samples are shown in Figure 1. The data for the standard sample is also shown in the figure. Clearly, the amount of material remove from the filter approaches a maximum for a total shake time of 4 min, as indicated by the graph. The error bars give a measure of the variability in the test data and are based on one standard deviation of the data. It is interesting to note the standard deviation for both samples after two minutes of shaking is quite low. It is doubtful, however, if any significance is associated with the observation. The figure shows that 4 min of shake time is adequate for removing most of the microspheres from the filters.

[1] Band pass of the narrow band filter was 435 – 445 nm.

[2] The sharp cut-off filter blocks all light of shorter wavelength. The characteristic wave length of a sharp-cut filter is defined as the wavelength at which the filter has a transmittance of 37%. The cut-off wavelength for the filter was 515 nm.

Table 1. Summary of filter weight and fluorometer data of experiment 1.

Sample No.	Sample Weight on Filter	Shake Time	Fluorometer Settings and Data				Normalized Fluorometer Reading/Sample Weight				Operator
			Uncorrected		Normalized for gain		*F*				
			Gain Setting of 50	Gain Setting of 10	Gain of 50 Data	Gain of 10 Data	Gain 50 data	Gain 10 data	Avg	Standard Deviation	
	g	min					g^{-1}	g^{-1}	g^{-1}		
S1a	0.36887	1	228	35	4.56	3.5	12.36	9.49	10.93	2.03	1
S1b	0.36887	2	238	48	4.76	4.8	12.90	13.01	12.96	0.08	1
S1c	0.36887	4	240	63	4.80	6.3	13.01	17.08	15.05	2.88	1
S1d	0.36887	6	270	57	5.40	5.7	14.64	15.45	15.05	0.56	1
S2a	0.36083	1	242	48	4.84	4.8	13.41	13.30	13.36	0.08	1
S2b	0.36083	2	245	50	4.90	5.0	13.58	13.86	13.72	0.20	1
S2c	0.36083	4	247	59	4.94	5.9	13.69	16.35	15.02	1.88	1
S2d	0.36083	6	260	52	5.20	5.2	14.41	14.41	14.41	0	1
reference	0.25045	NA	180	45	3.60	4.5	14.37	17.97	16.17	2.54	1

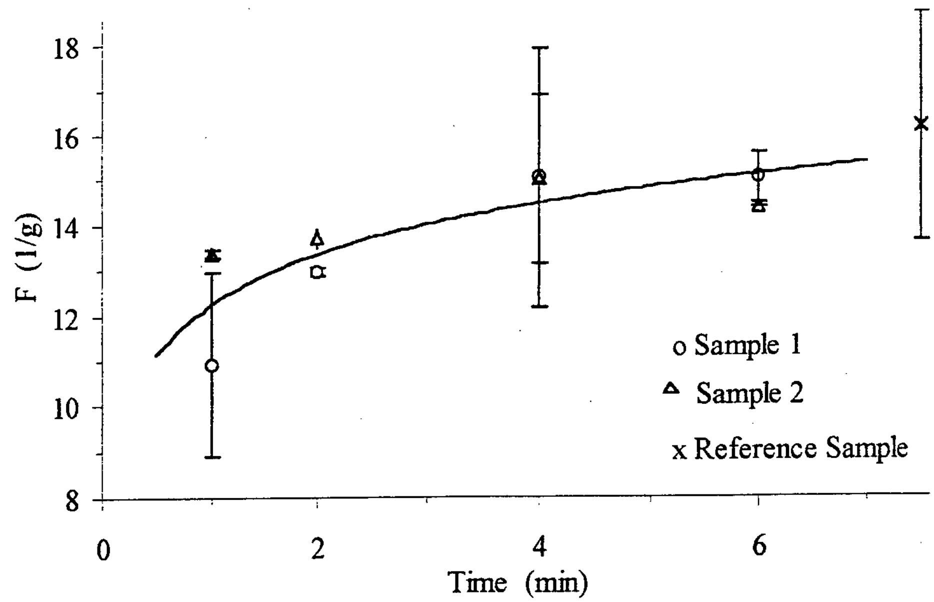

Figure 1. Normalized amount of microspheres removed from the filters as a function of total shake time. *F* refers to the normalized fluorometer reading divided by the sample mass deposited on the filter.

3.2. <u>Experiment 2, Effect of Operator Procedure</u>.

The data on the effect of the operator on the removal of PSL microspheres are summarized in Table 2 and plotted in Figure 2. Each operator examined four filters. The total shake time for each operator was 5 min. The data plotted in the figure is the average for the four filters. The error bar represents one standard deviation for the four readings. The data are compared with the data for the standard sample. Although there was more variation for the results from one of the operators, it is clear that the data compare reasonably close to the standard sample when the standard deviations are taken into account. No statistical tests were performed because of the small number of samples.

3.3. <u>Experiment 3, Effect of Fluorometer Settings</u>.

The data summarized in Table 2 are also plotted in Figure 3 to examine the two different gain settings. As in Figures 1 and 2, the data, for each gain setting, show reasonable agreement with the standard sample. However, both the test samples and the standard samples show a lack of instrument linearity.

Table 2. Summary of filter weight and fluorometer data of experiment 2

Sample No.	Sample Weight On Filter	Shake Time	Fluorometer Settings and Data				Normalized Fluorometer Reading/Sample Weight *F*				Operator
			Uncorrected		Normalized for Gain						
	G	min	Gain Setting of 50	Gain Setting of 10	Gain of 50 Data	Gain of 10 Data	Gain 50 data g^{-1}	Gain 10 data g^{-1}	Avg g^{-1}	Standard Deviation	
Filter S1-5	0.27421	5	147	28	2.94	2.8	10.72	10.21	10.47	0.36	1
Filter S2-5	0.35066	5	203	39	4.06	3.9	11.58	11.12	11.35	0.32	1
Filter S3-5	0.29423	5	160	30	3.20	3.0	10.88	10.20	10.54	0.48	1
Filter S4-5	0.31563	5	181	35	3.62	3.5	11.47	11.09	11.28	0.27	1
Filter S5-5	0.26054	5	153	28	3.06	2.8	11.74	10.75	11.25	0.71	2
Filter S6-5	0.41587	5	237	44	4.74	4.4	11.40	10.58	10.99	0.58	2
Filter S7-5	0.24816	5	147	26	2.94	2.6	11.85	10.48	11.16	0.97	2
Filter S8-5	0.19711	5	115	22	2.30	2.2	11.67	11.16	11.41	0.36	2
Reference 9	0.29178	NA	168	31	3.36	3.1	11.52	10.62	11.07	0.63	1
Reference 10	0.28133	NA	161	30	3.22	3.0	11.45	10.66	11.05	0.55	1
Reference 11	0.27775	NA	157	29	3.14	2.9	11.31	10.44	10.87	0.61	1
Reference 12	0.23736	NA	133	25	2.66	2.5	11.21	10.53	10.87	0.48	1
Reference 13	0.29007	NA	166	31	3.32	3.1	11.45	10.69	11.07	0.54	1
Reference 14	0.29037	NA	166	30	3.32	3.0	11.43	10.33	10.88	0.78	1

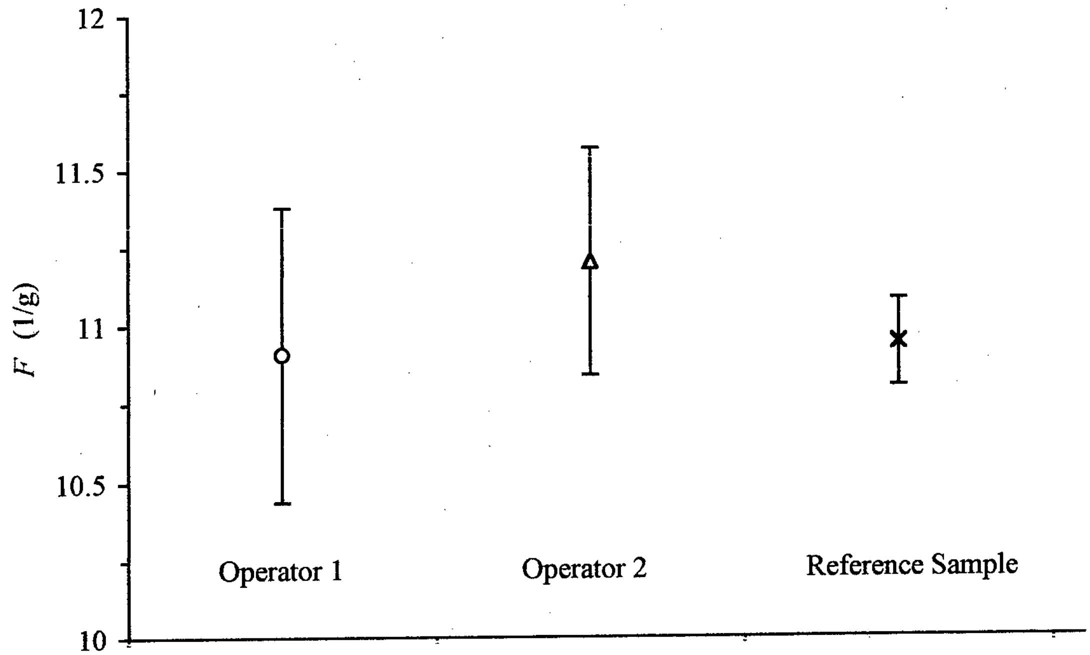

Figure 2. Normalized amount of microspheres removed from the filters based on two different operators performing the shaking.

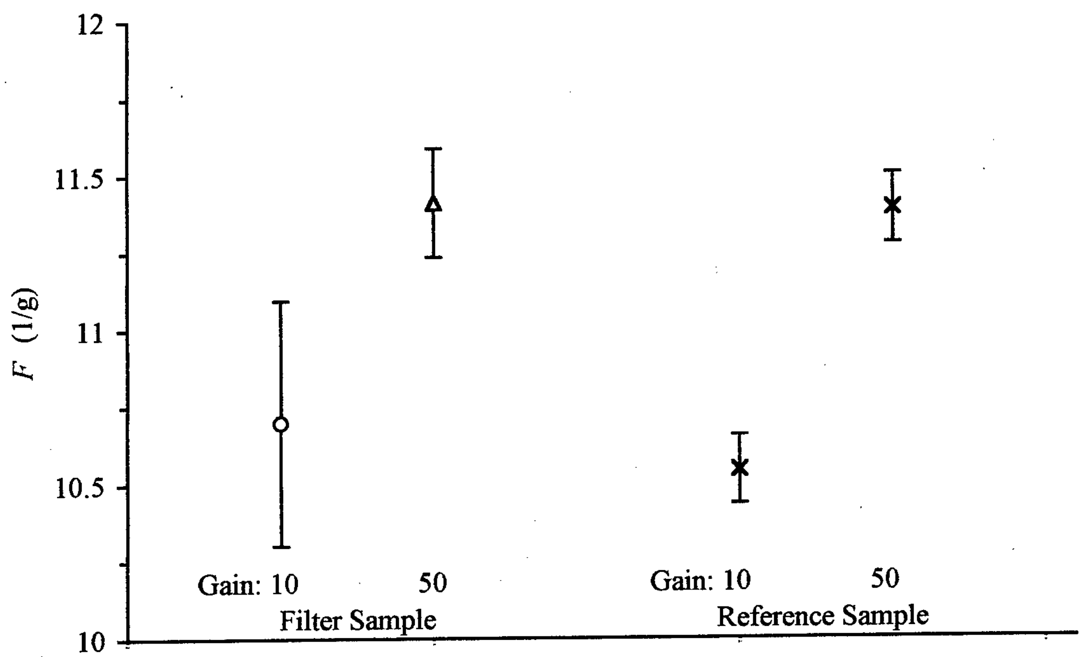

Figure 3. Effect of fluorometer gain settings on the recorded amount of normalized microspheres removed from the filters.

4. DISCUSSION

Although the data are considered to be reasonably consistent, there was some lack of repeatability. Possible causes may be due to re-zeroing the fluorometer and to the decay in the fluorescence of the PSL microspheres due to the exposure of the samples to room light may result in lower readings. Because of these problems, the data selected for comparison purposes were limited to measurements made with the same zero for each gain setting and made within a reasonable time frame.

Even though Experiment 1 shows that 4 min of shaking was adequate for removing most of the PSL microspheres from the filters, some prior data showed that even 6 min of vortexing, without hand shaking, was not sufficient to remove an acceptable level of PSL microspheres from the filter. Therefore, a shaking time of 5 min was chosen for our test procedure.

5. CONCLUSIONS

A satisfactory procedure for removing PSL microshperes from membrane filters was developed. The procedure consisted of inducing a vortex motion for 50 sec in a test tube containing the filter, glass beads, and the microspheres followed by 10 sec of manual shaking and repeating the sequence five times.

The results for the test procedure showed that there are minimal variations due to change in the experimentalist.

The fluorometer measurements that are compared should be taken with the same zero for each gain setting and made within a reasonable time frame.